with compliments R. C.

OBSERVATIONS ON THE PATHOGENIC YEASTS.

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OBSERVATIONS ON THE PATHOGENIC YEASTS.*

As we all know, yeast used to be classed as budding fungi or blastomycetes. This isolated them as a class, but afforded no indication of their relation to other fungi, so that between blastomycete and hyphomycete was an unknown gulf. Mid-Victorian mycologists suspected that certain yeasts which formed spores were more than blastomycetes, and that the spores were similar to the endospores of some ascomycetes. But this was the only link with other fungi till Hansen investigated the organisms which had figured so prominently in Pasteur's classical researches on fermentation. After adopting Lister's dilution method for securing pure cultures, Hansen's work was uninterrupted, and in ten years he made the yeasts better known than any other section of the fungi.

His study of film formation taught him a great truth, unsuspected by his predecessors, that in this film the yeast was growing in its mycelial stage in which it resembled the hyphomycetes. This transient, almost obsolete, phase of its life history enabled him to regard the active stage of fermentation as carried on by a primitive conidial or pseudoconidial phase, the so-called blastomycete or budding fungus. And he pointed out that in nature, as winter approached, this is completed by the stage of endosporulation. From perfect chaos there

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now emerged a perfect fungus showing mycelial, conidial (pseudoconidial) and sporogenous stages in every way comparable with a typical ascomycete. It only remained for Guilliermond to work out the cytology by demonstrating the presence of a perfect nucleus rapidly undergoing amitotic division during the pseudoconidial phase and slowly completing mitotic division at sporulation (which is often preceded by conjugation and is therefore the sexual stage).

The yeasts are now divided into two families, one of which is confined to tropical and Eastern countries, and the other containing our familiar yeasts consists of eight genera and many species. For details concerning these I can refer you to the text-books, one of the most interesting and complete being Guilliermond's *Les levures*, of which a new edition has lately been published in America. With few exceptions, however, it is not among the true yeasts of the text-books, but among depauperate forms that we find species pathogenic to man. As a matter of fact, some yeasts do not form endospores and therefore belong to the fungi imperfecti. In such yeasts the sporogenous stage has become obsolete as well as the mycelial stage, and there remains only a budding form which is not an entity but merely the pseudoconidial phase of an unknown fungus. This is a torula and as it represents the most familiar stage of brewery yeast it is said to grow in yeast conidial form.

In any fungus under pathogenic conditions, growth and dissemination proceed only by means of two primitive, non-quiescent forms—namely, yeast conidia and oidium-like elements (so-called because first studied in the old Oidium lactis) ; and the latter are necessarily preceded by mycelial growth but not the former. It is convenient to refer to these two elements

as pseudoconidia. So long as a ringworm remains under pathogenic conditions, growth and dissemination proceed entirely by means of oidial elements. An organism growing in this form only, even in culture, might be called oidiomyces. In torula under any conditions growth proceeds by yeast conidia and the organism is incapable of any other mode of growth—a blastomyces.

Pathogenic fungi may be arranged in two groups according as the mycelium is inconspicuous or distinct. In our transactions for 1921, my paper on ringworm deals with the latter group. We are now concerned only with the former—Mycelium inconspicuous: Pathogenic forms belong to torula and monilia.

(1) *Torula.*—Everything else having disappeared, the organism is fixed in budding form. Elongated cells there may be in some species, but these are not septated—at any rate, not multi-septated. Resting cells which are regarded as chlamydospores may occur in cultures.

Vuillemin (Guilliermond, op. cit. p. 473) has disinterred the old name cryptococcus to distinguish non-sporing pathogenic yeasts from torulas not known to be pathogenic Such mould-mongering is apt to lead to confusion.

(2) *Monilia.*—Whereas mycelium is obsolete in torula, in this genus there is primitive mycelium and growth proceeds by means of both pseudoconidial forms (oidial elements as well as yeast conidia). No other elements are found with the possible exception of chlamydospores in cultures.

The best-known species are the thrush fungi and these are remarkable for a tendency to discard more or less completely the mycelial and oidial form of growth and to confine themselves to the yeast conidial form. In short, thrush

fungi may appear even in culture either as monilias or reduced to torula form, though sub-culture on suitable media usually reveals the truth. Thus morphology may be misleading, and I regard the morphological distinctions proposed by De Beurmann and Gougerot (*Les nouvelles mycoses*, p. 27) as illusory. In practice, we must often rely mainly on sugar fermentation.

By way of illustration it may be useful to tabulate some forms met with in London in unhealthy conditions of the mouth and throat. Yeast and primitive mycelium were found in all.

	Glucose.	Galactose.	Sucrose.	Maltose.	Raffinose.	Dextrin.	Inulin.
1	—	—	—	—	—	—	—
2	+	—	—	—	—	—	—
3	+	—	—	+	—	—	—
4	+	—	—	+	+	—	+
5	+	—	+	+	—	+	—
6	+	+	+	—	+	—	+

Every fermenter of glucose acted on levulose and mannose. No fermenter of lactose, melibiose, mannit, arabinose or xylose was encountered. The sixth was not tried, but all the others liquefy wort or glucose gelatin in a month except the first, which may take a week longer.

The first is rare and belongs to the small group of non-fermenters. The whitish membrane on an inflamed tonsil was a tangled mass of this monilia. Its wort agar culture had a rugose surface. It does not film but in wort small floating whitish islets appear. Microscopically, it has numerous vacuoles which might be mistaken for spores. In ageing cultures sometimes turn pink and remain so in sub-cultures, so that possibly this is a parent of pink torulas.

This group is noteworthy because one member

is probably the parent of Klein's pathogenic yeast (*Path. Soc. Trans.*, 1901, p. 272), and of the closely related organism described by Ofenheim (*Lancet*, 1911, i., p. 659) in an ultimately fatal human infection. I examined the latter on various media over months without inducing it to leave the torula form. De Beurmann accepted it as a sporotrichum (*B.M.J.*, 1912, ii., 290) without any evidence whatever.

The second group (fermenters of hexose only) is commoner. It did not appear to be pathogenic and is mentioned because one form gives a fine inverted pine tree growth in wort gelatin punctures. The Americans think this is characteristic of their sprue fungus. I have also found the tree in a feeble lactose fermenter from milk. As a group they ferment feebly.

The third is the M. albicans group, the common London thrush fungus, which is often present in the mouths of adults. I have proved that the Sac. neoformans described by Sir K. Goadby as pathogenic in the gums is the torula form of this, and produced evidence that Mr. Foulerton's Sac. tumefaciens albus cannot be regarded as anything else (*B.M.J.*, 1913, ii., 1460). The interpretation of these as yeast forms of the thrush fungus is simple, rational and adequate. It also brings them into line with Ricketts' oidiomycosis of the skin (*Contributions* to *Medical Science*, Ricketts, pp. 59 and 148). The experimental methods of Ricketts and Foulerton were different, but their results were not markedly so. I put Ricketts' description in these words—a pathogenic, gelatin liquefying, maltose non-saccharose fermenting monilia in cultures of which yeast conidia predominate often and hyphæ are sometimes suppressed. Ricketts had a typical organism and Foulerton had a torula form.

The fourth, though a variant, belongs to the albicans group, and was found in the sputum and on the thickly furred tongue in a case of asthma in the adult. It is mentioned here because in my experience inulin fermenters are commoner than is generally believed.

The fifth belongs to the M. candida group, fully described and figured in every text-book of the fermentation industries. Its enzymes were further studied by Emil Fischer and P. Lindner. It is distinguished by its invertase being a strict endoenzyme ; by fermentation of saccharose, but not raffinose (it was long believed that the same enzyme split off levulose from either saccharose or raffinose) ; by fermentation of dextrin, which is not at all or only feebly fermented by European yeasts ; and by its preference for high temperatures (40° C.). Maltose is readily fermented, saccharose feebly. It was found in thrush in a child of six months.

In London, thrush is commonly due to M. albicans, but occasionally to other species, of which M. candida is best known. With the sugars in the table it will be seen that a series of thirty varieties may intervene between M. albicans as No. 1 and M. candida as No. 32. As a matter of fact, I have found five of these. And still there will be room for Ashford's sprue fungi.

The sixth form given in my table was found in the mucopurulent sputum of a baker suffering from bronchitis. Possibly it was a mouth contamination. Mycelial threads in cultures suggested a monilia, but the failure of a saccharose fermenting monilia to act on maltose was new to me. Accordingly a drop or two of the fermenting sucrose solution was added to boiling Fehling and reduction was instant. This ruled out monilial invertase and indicated

ordinary yeast invertase. If so, clearly it belonged to Hansen's second sub-group (saccharose non-maltose fermenters) of saccharomycetes. The fermentation of inulin but not dextrin facilitated its reference to Sac. marxianus. Its ability to grow and ferment at 45° C. and to produce numerous kidney-shaped spores at 20°—30° C., but not at 37° C. put the matter beyond doubt, as no other yeast is such a glutton for heat. Does this mean that, though its natural habitat is the grape, it passes part of the year as a harmless saprophyte in birds or mammals ?

In conclusion, I may say that many yeast infections have been described by dermatologists, especially by Prof. T. Caspar Gilchrist, of Baltimore, and by the late Dr. Howard T. Ricketts, of Chicago. In limiting my remarks to the organisms, I have pointed out how familiar fungi may masquerade under many aliases. And in my introduction I have tried to explain that if blastomyces is not wholly a myth it is hardly an entity and little more than a mode of growth. Just the same might be said of an oidiomyces, and probably an organism might be fixed in both forms. Finally, as the common London thrush fungus is similar to organisms found in American dermatomycoses, I think it probable that if we look for it in the way suggested by Ricketts, it will be found in granulomas of the skin in London also.